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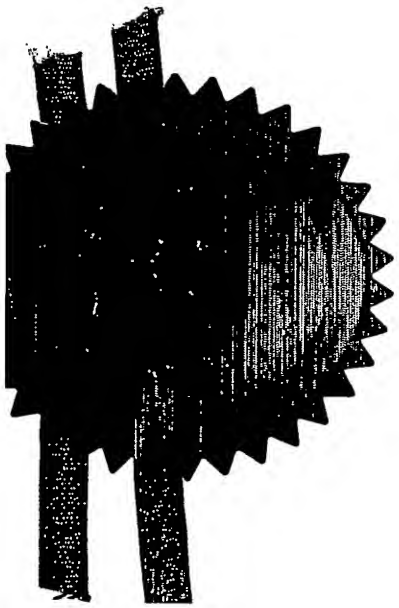

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2.	Patent application number *(The Patent Office will fill in this part)* 30 AUG 2002	**0220182.0**
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)* 08455628001 Patent ADP number *(if you know it)* If the applicant is a corporate body, give the country/state of its incorporation	**CARDIOVASCULAR RESEARCH INSTITUTE MAASTRICHT, UNIVERSITY OF MAASTRICHT** **PO BOX 616** **6200 MD MAASTRICHT** **THE NETHERLANDS** **THE NETHERLANDS**
4.	Title of invention	**Organic compounds**
5.	Name of your agent *(If you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* Patents ADP number *(if you know it)*	Novartis Pharmaceuticals UK Ltd Patents and Trademarks Wimblehurst Road HORSHAM West Sussex RH12 5AB Patents ADP No. 07183220001

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B. A. Yorke & Co

B.A. Yorke & Co. **30 August 2002**

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New Use

The present invention concerns the use of vitamin K and derivatives thereof to prevent or treat a reduction in elasticity and distensibility of the vasculature, and thereby to lower blood pressure and prevent cardiovascular disease.

Background

The process of aging is associated with irreversible physiological changes to the circulatory system, leading to an increased risk of blood pressure disorders, Coronary Heart Disease (CHD), and stroke. For women, this risk rises dramatically after the onset of menopause. These conditions have a significant impact on quality of life for the middle-aged and elderly and account for a large proportion of deaths and chronic illnesses in modern societies.

Patients suffering from cardiovascular disorders are frequently prescribed anticoagulants, antihypertensives, cholesterol-lowering medications, and the like. These medications usually present harmful side-effects or health risks, and moreover, the chronic effects of taking such medication regularly over the course of years or decades are not well studied. As life expectancies increase, it would be desirable to find long-term, safe and reliable natural therapies to prevent, treat or even reverse the consequences of aging on the vasculature.

Changes in mechanical properties of the main arteries have major implications for the development of vascular disease. Arteries, especially the larger elastic arteries such as the common carotid artery, become stiffer with age. Peak elasticities are achieved at about age 14-15, after which they deteriorate gradually. Measures of large artery stiffening include compliance and distensibility. Compliance reflects the buffering capacity of the vascular vessel wall, and distensibility refers to the intrinsic vascular wall elasticity. In cross-sectional studies it has been shown that the distensibility and compliance of the elastic common carotid artery decrease linearly with age. The increase in arterial stiffness with increasing age is suggested to occur more rapidly in women aged between 45 and 60 years than in men of the same age group due to the lack of oestrogen after menopause.

Reductions in complicance and distensibility result in an impairment of the arterial system to cushion pulsatile pressure. Arterial stiffening results in a higher pulse wave velocity and

earlier wave reflections. This increases systolic and pulse pressure and consequently cardiac workload. To compensate, the arterial diameter increases with age. Over time, arterial stiffening can contribute to the development of *inter alia* left ventricular hypertrophy, congestive heart failure and coronary heart disease.

It has long been recognized that vitamin K is an essential component of the diet. It was first identified as an element needed to prevent haemorrhaging by activating blood-clotting factors. Natural K-vitamers are menadione-derivatives differing from each other in the polyisoprenoid side chain attached to the 3-position of the ring structure. Vitamin K can be provided in the diet by dark green, leafy vegetables (K_1, or phylloquinone), and is synthesized in the small intestine by resident symbiotic bacteria (K_2, or menaquinone). Vitamin K is also needed for carboxylation of two bone matrix proteins necessary for normal bone metabolism. In EP679394 it is disclosed that a high dietary intake of vitamin K and related molecules can reduce arterial calcification, from which it is concluded that arteriosclerosis can be treated using vitamin K.

Studies have shown that age-related stiffening of the arteries can be distinguished from arteriosclerotic/atherosclerotic calcification. Whereas atherosclerosis is invariably associated with inflammation and starts with destruction of the endothelial cell layer at the luminal side of the tunica intima, age-related stiffening is a process which originates in the tunica media, and is not associated with inflammation. It is believed that age-related stiffening occurs as a result of deposition of minerals around the elastic fibres of the tunica media, followed by degradation of the elastin structure. After deterioration of the elastin, the elastic properties of the artery depend on collagen, which is much less flexible.

For the first time, it has now been shown that arterial compliance and distensibility can be improved long-term by administering vitamin K, relative to subjects receiving no nutritional supplementation (placebo). Thus, administration of vitamin K is a useful therapeutic measure to prevent the development of cardiovascular disease conditions including hypertension, left ventricular hypertrophy, congestive heart failure, myocardial infarction, stroke, Mönckeberg's sclerosis and coronary heart disease.

Summary of the Invention

In a first aspect, the invention provides use of a composition comprising vitamin K or a derivative thereof, optionally together with vitamin D or a derivative thereof, in the manufacture of a medicament or nutritional formulation for treating or preventing age-related stiffening of arteries.

In a second aspect, the invention provides use of a composition comprising vitamin K or a derivative thereof, optionally together with vitamin D or a derivative thereof, in the manufacture of a medicament or nutritional formulation for treating or preventing an age-related decrease in compliance and/or distensibility of arteries and/or an age-related increase in pulse pressure.

In another aspect, the invention provides use of a composition comprising vitamin K or a derivative thereof, optionally together with vitamin D or a derivative thereof, in the manufacture of a medicament or nutritional formulation for treating or preventing any of: hypertension, left ventricular hypertrophy, congestive heart failure, myocardial infarction, stroke, Mönckeberg's sclerosis, and coronary heart disease.

In a further aspect, the invention provides a composition for promoting healthy arteries, comprising vitamin K or a derivative thereof, and optionally vitamin D or a derivative thereof, and one or more additional components selected from: polyphenols, vitamin C, vitamin E (tocopherols and/or tocotrienols), L-Arginine, phytosterols, antihypertensive peptides, soluble fibers (e.g. guar, pectin), omega-3, omega-6 and/or omega-9 fatty acids, carnitine, taurine, coenzyme Q10, creatine, folic acid, folates, magnesium, potassium, vitamin B6, and vitamin B12.

In another aspect of the invention there is provided a composition for promoting healthy arteries which comprises: 0.5-1.5mg vitamin K; 5-10μg vitamin D; 450-550mg Calcium; 7-12 mg Zinc; and 100-200mg Magnesium.

In yet another aspect of the invention there is provided a kit comprising Vitamin K or a derivative thereof, and optionally vitamin D or a derivative thereof and a medicament, for simultaneous, separate or sequential administration, wherein said medicament is selected from the group consisting of: anticoagulants, antithrombotics, fibrinolytics, antihypertensives,

diuretics, antianginals, hypolipidaemic agents, beta-blockers, ACE inhibitors, cardiac glycosides, phosphodiesterase inhibitors, antiarrhythmics, and calcium antagonists.

Description of the Figures

Figure 1 shows how the Distensibility Coefficient (DC) varies over a 3 year study period when placebo, Vitamin D (MD) and Vitamins K plus D (MDK) are administered to a group of postmenopausal women.

Figure 2 shows how the Compliance Coefficient (CC) varies over a 3 year study period when placebo, Vitamin D (MD) and Vitamins K plus D (MDK) are administered to a group of postmenopausal women.

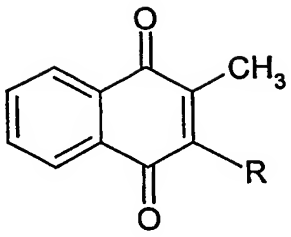
In each case the black bar represents the baseline measurement (100%), and the shaded bars are the % change relative to baseline after 3 years.

Detailed Description of the Invention

This invention provides the first form of directed therapy for reducing age-related arterial stiffening (as distinct from stiffening due to atherosclerosis). Arterial elastic properties (compliance and distensibility) deteriorate with age. However, the severity of this downward trend was found to be significantly reduced in a group of menopausal women who regularly consumed a supplement of vitamin K (plus Vitamin D) over the course of 3 years. These women were selected for the study on the basis of criteria which included a lack of evidence of atherosclerotic disease and low risk factors for the disease.

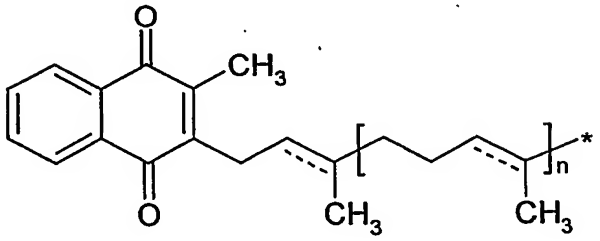
Preferrably vitamin K and derivatives refers to one or more compounds of Formula 1', and/or their pharmaceutically or nutritionally acceptable salts,

Formula 1'



where R may be any covalently linked organic group including polyisoprenoid residues, esters, ethers, thiol adducts, etc.

and especially



in which n is an integer from 1 to 12;
and in which the broken lines indicate the optional presence of a double bond.

Vitamin K and derivatives thereof, as used herein, refers in particular to phylloquinone (also known as vitamin K_1), dihydrophylloquinone; menaquinone-4 (MK-4) and the long chain menaquinones. It is generally accepted that the naphthoquinone is the functional group, so that the mechanism of action is similar for all K vitamins. Differences may be expected, however, with respect to intestinal absorption, transport, tissue distribution, and bioavailability. For use in the present invention, phylloquinone and MK-4 are preferred, and phylloquinone is particularly preferred.

Sources of vitamin K which can be used according to the present invention include the following: phylloquinone from natural sources such as vegetable extracts, fats and oils, synthetic phylloquinone, synthetic vitamin K3 (menadione), different forms of vitamin K2: synthetic MK-4, MK-5, MK-6, MK-7, MK-8, MK-9, MK-10, MK-11, MK-12 and MK-13, natto

(food prepared from fermented soy-bean, rich in MK-7), and other fermented foods or dairy products.

The dose of vitamin K useful in performing the invention is not restricted but varies depending on, for example, the age of the subject and the degree of risk of developing arterial stiffening. Current AI values or Adequate Intakes (as determined by the Institute of Medicine) are 120 µg for men and 90 µg for women. Benefits may be derived by selecting dosages higher than the AI values, particularly in population groups where vitamin K deficiencies are common, for instance among postmenopausal women. For example, suitable dosages may lie in the range 10 to 1000 µg, more preferably 50 to 500µg, and most preferably 100 to 200 µg vitamin K/day. Where national legislation permits, it may be advisable to provide dosage ranges as high as from 1 to 200mg/day, preferably from 5 to 150mg/day, and more preferably from 10 to 100 mg/day. No upper limit (UL) has been defined for vitamin K, since it is not known to have any adverse effects on the body.

In terms of body weight, daily dosage may vary between 0.5 to 50 µg/kg body weight/day, preferably 0.75 to 25 µg/kg body weight/day, more preferred 1 to 15 µg/kg body weight/day.

Vitamin D is included together with vitamin K in the composition used in the clinical study, and may play a role in supporting the function of vitamin K in preventing arterial stiffening. Any form of natural or synthetic vitamin D may be employed, including vitamin D_1, vitamin D_2 (calciferol), vitamin D_3 (cholecalciferol) and vitamin D analogues (e.g. alfacalcidol, dihydrotachysterol, calcitriol). Natural sources of vitamin D include saltwater fish, organ meats, fish-liver oils and egg yolk. Suitable dosages of vitamin D are 2 to 50 µg/day, preferably 5 to 20µg/day, and most preferably about 7 to 10µg/day.

In the clinical study described in the Examples arterial wall property measurements were taken at t=0 and t=3 years. This is good support for concluding that ingestion of vitamin K over long periods is an effective way of limiting an increase in arterial stiffness. The preferred treatment period is a minimum of 6 months, more preferably at least 18 months, and ideally at least 36 months. In fact, as there are no adverse side-effects associated with dietary vitamin K supplementation, it should be regarded as an essential component of a healthy lifestyle over the course of a lifetime, and especially throughout middle age and old age.

The preferred route of administration of vitamin K is enterally, especially orally, but the parenteral or topical routes are viable alternatives. Vitamin K is conventionally provided in the form of tablets or capsules, i.e. in a pharmaceutical or dietary supplement format. For pharmaceutical preparations or dietary supplements the vitamin K may be compounded with pharmaceutically acceptable carriers, excipients or diluents in the forms of pills, tablets (coated or uncoated), hard or soft capsules, dragées, lozenges, oral solutions, suspensions and dispersions, syrups or sterile parenteral preparations. Suitable excipients include inert diluents such as calcium carbonate, sodium carbonate, lactose, calcium phosphate, sodium phosphate; granulating and disintegrating agents such as cornstarch or alginic acid; binding agents such as starch gelatin or acacia; effervescents; and lubricating agents such as magnesium stearate, stearic acid or talc.

It is also possible to deliver Vitamin K (optionally together with vitamin D) in a fortified food or beverage product. Preferred nutritional product formats include: juice drinks, dairy drinks, powdered drinks, sports drinks, mineral water, soy beverages, hot chocolate, malt drinks, biscuits, bread, crackers, confectioneries, chocolate, chewing-gum, margarines, spreads, yoghurts, breakfast cereals, snack bars, meal replacements, protein powders, desserts, and medical nutrition tube feeds and nutritional supplements.

Conventional additives may be included in the compositions of the invention, including any of those selected from preservatives, chelating agents, effervescing agents, natural or artificial sweeteners, flavoring agents, coloring agents, taste masking agents, acidulants, emulsifiers, thickening agents, suspending agents, dispersing or wetting agents, antioxidants, and the like.

As consumers who are at risk from stiffening arteries are also inclined to develop other ageing-related disorders, it may be of benefit to combine vitamin K (and optionally vitamin D) with other healthy or pharmaceutically active components in a single composition, or in the form of a kit for simultaneous, sequential or separate administration. For instance, it is envisaged that vitamin K could be provided in conjunction with medicaments selected from anticoagulants such as aspirin or COX-2 inhibitors, antithrombotics, fibrinolytics, antihypertensives, diuretics, antianginals, hypolipidaemic agents including statins, bile acid sequestrants, nicotinic acid derivatives, and fibrates, beta-blockers, ACE inhibitors, cardiac

glycosides, phosphodiesterase inhibitors, antiarrhythmics, and calcium antagonists. Other bioactive substances for co-administration include: polyphenols, vitamin C, vitamin E (tocopherols and/or tocotrienols), L-Arginine, phytosterols, antihypertensive peptides, soluble fibers (e.g. guar, pectin), omega-3, omega-6 and/or omega-9 fatty acids, carnitine, taurine, coenzyme Q10, creatine, folic acid, folates, magnesium, potassium, vitamin B6, and vitamin B12.

Anyone perceived to be at risk from cardiovascular disorders or already suffering from conditions such as angina pectoris, hypertension, a history of stroke, and other cerebrovascular disorders can benefit from ingesting vitamin K in order to counteract age-related stiffening of the arteries. Particular target population groups are: postmenopausal women, diabetics, obese individuals, smokers, alcoholics, sedentary and inactive people, the elderly, hemodialysis patients, men over 40 years of age, people suffering from chronic stress, and those consuming an unhealthy diet prone to causing cardiovascular diseases.

Although it is believed that vitamin K is effective at limiting age-related stiffness throughout the network of arteries in the body, its therapeutic effect on the body is probably most significant with respect to its influence on the larger elastic arteries of the body, especially the common carotid arteries supplying blood to the neck and head, the aorta, and the renal arteries.

By reducing arterial stiffening, vitamin K also has the effects of counteracting the sequelae of arterial stiffening, namely hypertension, left ventricular hypertrophy, congestive heart failure, myocardial infarction, stroke and coronary heart disease. "Elevated blood pressure" or "hypertension" as used herein refers to a blood pressure persistently exceeding 140/90 mmHg (systolic/diastolic).

Examples

Clinical Study to compare effects of supplementation of Vitamin D and Vitamin D + Vitamin K on a group of healthy postmenopausal women.

Subjects

The participants were enrolled in a 3-year double-blind placebo-controlled clinical trial in which the effects of minerals, vitamin D- and vitamin K-containing supplements were investigated on bone mineral density and vessel wall characteristics. Inclusion criteria were: apparently healthy women, Caucasian, between 50 and 60 years old, and at least 2 years postmenopausal. Exclusion criteria were: use of oral anticoagulants, corticosteroids, hormone replacement therapy, vitamin concentrates or food supplements, and high alcohol consumption (> 6 glasses/day). In total 181 women met the criteria for participation and were randomized into the study. Information on cardiovascular risk factors, current health status, medical history, drug use and smoking behaviour was collected before the start of the study. Within this trial participants underwent clinical examinations at 0, 3, 12, 18, 24 and 36 months. The vascular examinations took place at baseline and at the end of the study after 3 years.

All participants gave written informed consent and the trial was approved by the Maastricht University Hospital Medical Ethics Committee.

Study Design

The subjects were randomized into three groups. In the first group (n=60) participants received a placebo (maltodextrin), in the second group (n=58) participants received a supplement containing 500 mg calcium (natural calcium complex derived from milk), 10mg zinc, 150 mg magnesium and 8 µg vitamin D_3 (minerals + vitamin D = MD-group), and in the third group (n=63) participants received a supplement containing the same constituents as the MD group but with an additional 1mg of vitamin K1 (minerals + vitamins D+K = MDK-group). The three different types of supplements were similar in appearance and taste, and participants were allowed to choose between a supplement in the form of a tasteless powder (to be mixed with water before intake) or in the form of chocolate-coated tablets with a crunchy malt core. Participants were instructed to take one sachet with powder or three tablets per day during evening hours, preferably after the meal. Also, they were advised to maintain their usual diets and to avoid taking supplements containing either calcium, vitamin

D, or vitamin K for two months before and throughout the study. Novartis Consumer Health SA (Nyon, Switzerland) prepared and provided all supplements.

The right common carotid artery of each patient was investigated. The same investigator performed all examinations at the start and the end of the study and for each participant several repeated measurements (5-7) are made during one session. Reproducibility was evaluated for assessment of common carotid artery distension and diameter.

Before the vascular examination, height and weight were measured with standardized equipment to estimate the body mass index (weight/height^2).

Measurements

The primary outcome measures for the purposes of this study were the vessel wall characteristics of the common carotid artery measured with ultrasound.(ATL Mark V). The ultrasonic vessel wall tracking system (WTS) to determine arterial wall properties has been described in detail before (Hoeks AP et al., *Ultrasound Med Biol* 1990; 16:121-8, and Kool MJF et al., *Cardiovascular Research* 1994;28:610-614). This ultrasound system provides estimates of the arterial end-diastolic diameter (d) and the change in diameter from diastole to systole (Δd) normalized for the end-diastolic diameter ($\Delta d/d$) for each captured heart beat. In parallel with diameter change measurement, arterial blood pressure was recorded at the level of the brachial artery by means of a semiautomated oscillometric device (DINAMAP). Pulse pressure (Δp), defined as systolic minus diastolic blood pressure, was determined by averaging the three measurements nearest to the distension measurements. From d, Δd and Δp, vascular distensibility (DC) and compliance (CC) were calculated according to the following equations:

$DC = (2d\Delta d + \Delta d^2)/(d^2/\Delta p)$ (Distensibility Coefficient)

$CC = \pi(2d\Delta d + \Delta d^2)/4\Delta p$ (Compliance Coefficient)

The intima-media thickness (IMT) of the posterior wall was measured simultaneously at the same location (2-3 cm proximal to the bifurcation) of the common carotid artery where the diameter and diameter changes were measured. At the end of the session, recorded IMT-files are processed employing the wall thickness program. The threshold for the derivative

was maintained at 0.025. Each heart-beat within a recording resulted in an estimate of wall thickness; the median of the estimates per recording was used for further evaluation.

Statistical Analysis

Statistical analysis was performed using the Statistical Package SPSS (SPSS Corp, Chicago, IL). Results are presented as means ± standard deviation (SD), unless indicated otherwise. Only participants who had completed the study were included in the analysis. Furthermore, participants who during the study had started to use medications which are known to have a direct effect on the vessel wall, were excluded from analysis. Also, participants with atherosclerotic plaques in the common carotid artery and a high variability in the results (arterial translation of > 2 mm and beat-to-beat variation in distension of > 20%) were excluded.

A paired t-test was used to evaluate the change in the vessel wall characteristics over the three years within each group. We considered a level of $p<0.05$ to be statistically significant. For every participant, the percentage change from baseline in all parameters was calculated and the mean change from baseline was calculated per group. Primary outcome analysis consisted of comparison of the change in DC, CC, PP and IMT between the MD-group and placebo and between the MDK-group and placebo. Linear regression analysis was used with the change in vascular parameters relative to baseline as dependent variable and the treatment groups and several covariates as explanatory variables. Baseline values of age, BMI, smoking (yes or no), heart rate and mean arterial pressure were chosen as covariates, because their influence on the change in vascular properties or response to the supplementation could not be excluded.

Vascular parameters of elasticity

Table 1 details the baseline measurements of each study group. Table 2 summarizes per group the differences between the mean values at baseline and at the end of the study for all vascular parameters with their paired-levels of significance. As was to be expected, the DC and CC in the placebo group decreased significantly (by 10% and 6%, respectively). The PP, on the other hand, increased by 7%, but the increase did not reach the level of significance. In the MD-group, DC decreased significantly (by 7%) and CC decreased by 4%, while the PP increased by 6%; however these latter two changes did not reach the level of

significance. In the MDK-group, however, the DC and CC remained approximately constant over the three year period, the CC even showing a tendency to increase (+3%). The PP remained unchanged throughout the entire study period.

Figures 1 and 2 (see also Table 3 and Table 3a) illustrate the percentage change in DC and CC respectively of the three groups. After adjustment for baseline heart rate, mean arterial pressure, age, weight and smoking, the changes in the placebo- relative to the MDK-group remained statistically significant and were: 8.8% decrease of DC (95% CI: 1.9 to 21.4), 8.6% decrease of CC (95% CI: 1.8 to 20.3), and 6.3% increase of PP (95% CI: -17.1 to –0.7). In the same analysis no differences were found between the changes in the placebo- and the MD-group: 2.5% decrease of DC (95% CI: -14.8 to 6.3), 2.2% decrease of CC (95% CI: -13.8 to 6.3), and 0.11% increase of PP (95% CI: -5.6 to 12.1).

Discussion of Results

The deleterious effects on the arteries of aging over a period of 3 years are clearly evident from the control (placebo) group, and underline how rapidly the vasculature can go into decline. The medical practitioner, being aware of the link between a decrease in elasticity of the arteries and diverse cardiovascular conditions, would recognise from these data that there is an urgent need to find a treatment method capable of combating the rapid decline in arterial elasticity, particularly in postmenopausal women.

The MD group, who received a vitamin D supplement, failed to show any improvement in measures of vascular wall aging relative to the placebo group. It can be concluded that provision of vitamin D alone is not capable of delivering cardiovascular benefits to postmenopausal women fulfilling the criteria applied in the present study.

In stark contrast to the placebo and MD groups, the MDK group showed significant relative improvements in distensibility, compliance and pulse pressure over the 3 year period of the study. These results demonstrate that regular consumption of vitamin K, or of the combination of vitamin K and vitamin D, can slow and maybe even reverse the process of stiffening of the arteries. As a consequence of slowing down the process of arterial stiffening, vitamin K supplementation inevitably impacts on the incidence of cardiovascular disorders linked to arterial stiffening, including those related to increased strain on the heart

and reduced responsiveness of the circulatory system to changes in demand.

Table 1: Baseline characteristics (mean ± standard deviation) in the three treatment groups

Baseline-characteristics	**Placebo** (n=40)	**MD-group** (n=30)	**MDK-group** (n=38)
	Mean ± SD	Mean ± SD	Mean ± SD
Age (yr)	54.1 ± 3.0	55.9 ± 2.8*	55.4 ± 2.8
Weight (kg)	69.5 ± 11.9	70.6 ± 11.1	66.3 ± 9.5
Height (m)	1.65 ± 0.05	1.65 ± 0.07	1.63 ± 0.06
BMI (kg/m^2)	25.6 ± 4.3	26.0 ± 4.4	25.1 ± 3.1
Postmenopausal age (yr)	4.6 ± 3.7	7.6 ± 5.1**	5.1 ± 4.3
non-smokers (%)	75.0	73.9	85.0
Diameter (µm)	7162 ± 562	7314 ± 582	7173 ± 411
Distension (µm)	372 ± 118	353 ± 83	332 ± 83
Pulse Pressure (mmHg)	51.9 ± 11.1	52.9 ± 10.1	53.7 ± 14.3
Heart Rate (beats/min)	60.8 ± 9.2	63.1 ± 8.9	60.6 ± 6.6
CC (mm^2/kPa)	0.64 ± 0.23	0.61 ± 0.20	0.56 ± 0.17
DC (MPa^{-1})	15.8 ± 5.2	14.5 ± 4.0	14.0 ± 4.0
IMT (mm)	0.63 ± 0.11	0.64 ± 0.10	0.61 ± 0.08

* significant different from placebo ($p<0.05$)

** significant different from placebo and MDK-group ($p<0.05$)

Table 2: Change in vessel wall characteristics (mean ± SD) in study population after 3 years

Vessel wall characteristics	**Placebo** (n=40) Difference between T=0 and T=3 years (paired t-test)	**MD-group** (n=30) Difference between T=0 and T=3 years (paired t-test)	**MDK-group** (n=38) Difference between T=0 and T=3 years (paired t-test)
Diameter (μm)	196 ± 295 (p=0.00)	154 ± 179 (p=0.00)	131 ± 226 (p=0.00)
Distension (μm)	-21 ± 61 (p=0.03)	-12.6 ± 47 (p=0.15)	-3.9 ± 49 (p=0.63)
Pulse Pressure (mmHg)	2.7 ± 9.9 (p=0.09)	2.8 ± 10.1 (p=0.14)	-0.18 ± 7.6 (p=0.89)
DC (MPa^{-1})	-1.8 ± 3.4 (p=0.00)	-1.4 ± 3.0 (p=0.02)	-0.39 ± 3.0 (p=0.43)
CC (mm^2/kPa)	-0.05 ± 0.1 (p=0.01)	-0.04 ± 0.11 (p=0.10)	0.01 ± 0.11 (p=0.75)
IMT (mm)	0.05 ± 0.08 (p=0.00)	0.02 ± 0.09 (p=0.32)	0.06 ± 0.06 (p=0.00)
Heart rate (beats/min)	3.0 ± 7.0 (p=0.01)		

Table 3: Mean % change from baseline in vessel wall characteristics.
(for each subject the % change from baseline is calculated for each variable and then the mean of these individual changes is calculated per group)

Vessel wall characteristics	**Placebo** (n=40) Mean % change from baseline	**MD-group** (n=30) Mean % change from baseline	**MDK-group** (n=38) Mean % change from baseline
Diameter (μm)	2.8% ± 4.1	2.2% ± 2.5	1.8% ± 3.1
Distension (μm)	-4.3% ± 15.9	-2.4% ± 13.0	0.3% ± 15.9
Pulse Pressure (mmHg)	6.5% ± 19.7	6.3% ± 20.0	0.2% ± 13.4*
DC (MPa^{-1})	-9.6% ± 21.4	-7.1% ± 18.3	-0.8% ± 21.9*
CC (mm^2/kPa)	-5.9% ± 19.5	-3.7% ± 18.6	2.7% ± 20.4*
IMT (mm)	8.6% ± 13.5	4.0% ± 13.9	9.8% ± 9.8

*significant difference with placebo after adjustment for age, weight, smoking, mean arterial pressure and heart rate (linear regression analysis table 3a)

Table 3a: Multivariate regression analysis of the effects of the MDK-group and the MD-group compared to placebo on the change in vessel wall characteristics after three years with the following covariables: baseline age, weight, smoking, heart rate and mean arterial pressure.

Variables	Coefficient ± SEM	P	95% CI
Y= change in DC (% relative to baseline)			
X= MDK	11.7 ± 4.9	0.020	1.9 to 21.4
X= MD	4.2 ± 5.3	0.430	-6.3 to 14.8
Y=change in CC (% relative to baseline)			
X= MDK	11.1 ± 4.7	0.019	1.8 to 20.3
X= MD	3.8 ± 5.0	0.459	-6.3 to 13.8
Y= change in PP (% relative to baseline)			
X= MDK	-8.9 ± 4.1	0.034	-17.1 to -0.70
X= MD	-3.3 ± 4.5	0.465	-12.1 to 5.6
Y= change in IMT (% relative to baseline)			
X= MDK	3.0 ± 3.1	0.345	-3.23 to 9.15
X= MD	-2.4 ± 3.3	0.476	-8.9 to 4.2

Claims

1. Use of a composition comprising vitamin K or a derivative thereof, optionally together with vitamin D or a derivative thereof, in the manufacture of a medicament or nutritional formulation for treating or preventing age-related stiffening of arteries.

2. Use of a composition comprising vitamin K or a derivative thereof, optionally together with vitamin D or a derivative thereof, in the manufacture of a medicament or nutritional formulation for treating or preventing an ageing-related decrease in compliance and/or distensibility of arteries and/or an ageing-related increase in pulse pressure.

3. Use of a composition according to claim 1 where in said artery stiffening results from calcification of the tunica media.

4. Use of a composition comprising vitamin K or a derivative thereof, optionally together with vitamin D or a derivative thereof, in the manufacture of a medicament or nutritional formulation for treating or preventing any of: hypertension, left ventricular hypertrophy, congestive heart failure, myocardial infarction, stroke, Mönckeberg's sclerosis and coronary heart disease.

5. Use according to any of claims 1 to 4, wherein said vitamin K is vitamin K_1 (phylloquinone) or Vitamin K_2 (menaquinone).

6. Use according to claim 5 wherein said vitamin K is vitamin K_1 (phylloquinone).

7. Use according to any of claims 1 to 6 wherein the daily dosage of vitamin K or a derivative thereof is in the range 50µg-1000µg.

8. Use according to any of claims 1 to 7 wherein the medicament or nutritional composition comprises vitamin D or a derivative thereof.

9. Use according to claim 8 wherein said vitamin D is vitamin D_3 (cholecalciferol).

10. Use according to any of claims 1 to 9 wherein the medicament or nutritional formulation is for administration to a postmenopausal woman.

11. Use according to any of claims 1 to 10 wherein the medicament or nutritional formulation is to be administered over a period of at least 12 months, preferably at least 36 months.

12. Use according to claim 1 or claim 2 wherein said arteries are the common carotid arteries.

13. A composition for promoting healthy arteries, comprising:
(a) vitamin K or a derivative thereof; and
(b) one or more additional components selected from: polyphenols, vitamin C, vitamin E (tocopherols and/or tocotrienols), L-Arginine, phytosterols, antihypertensive peptides, soluble fibers (e.g. guar, pectin), omega-3, omega-6 and/or omega-9 fatty acids, carnitine, taurine, coenzyme Q10, creatine, folic acid, folates, magnesium, potassium, vitamin B6, and vitamin B12; and
(c) optionally, vitamin D or a derivative thereof.

14. A composition according to claim 13 which comprises in a single dose: 0.5-1mg vitamin K and 5-10μg vitamin D.

15. A composition for promoting healthy arteries which comprises: 0.5-1.5mg vitamin K; 5-10μg vitamin D; 450-550mg Calcium; 7-12 mg Zinc; and 100-200mg Magnesium.

16. A composition according to claim 15 which comprises about 1mg Vitamin K_1; 8 μg vitamin D_3, 500mg Calcium, 10mg Zinc; and 150mg Magnesium.

17. A composition according to any of claims 13 to 16 which is a food or beverage product or a dietary supplement.

18. A kit comprising Vitamin K or a derivative thereof, and optionally vitamin D or a derivative thereof, and a medicament, for simultaneous, separate or sequential administration, wherein said medicament is selected from the group consisting of:

anticoagulants, antithrombotics, fibrinolytics, antihypertensives, diuretics, antianginals, hypolipidaemic agents, beta-blockers, ACE inhibitors, cardiac glycosides, phosphodiesterase inhibitors, antiarrhythmics, and calcium antagonists.

19. A method of preventing or treating age-related arterial stiffening, hypertension, left ventricular hypertrophy, congestive heart failure, myocardial infarction, stroke, Mönckeberg's sclerosis or coronary heart disease, comprising administering to a person in need of such treatment an effective amount of vitamin K or a derivative thereof and optionally vitamin D or a derivative thereof.

Abstract

Vitamin K is effective in counteracting the reduction in arterial elasticity normally associated with the aging process. A pharmaceutical composition or nutritional formulation comprising vitamin K can be used to combat age-related stiffening of the arteries, and the consequences thereof, namely pulmonary congestion, hypertension, left ventricular hypertrophy, congestive (right sided) heart failure, left sided or left ventricular failure, chronic cardiac failure, angina pectoris, myocardial infarction, Mönckeberg's sclerosis and stroke.

Fig. 1

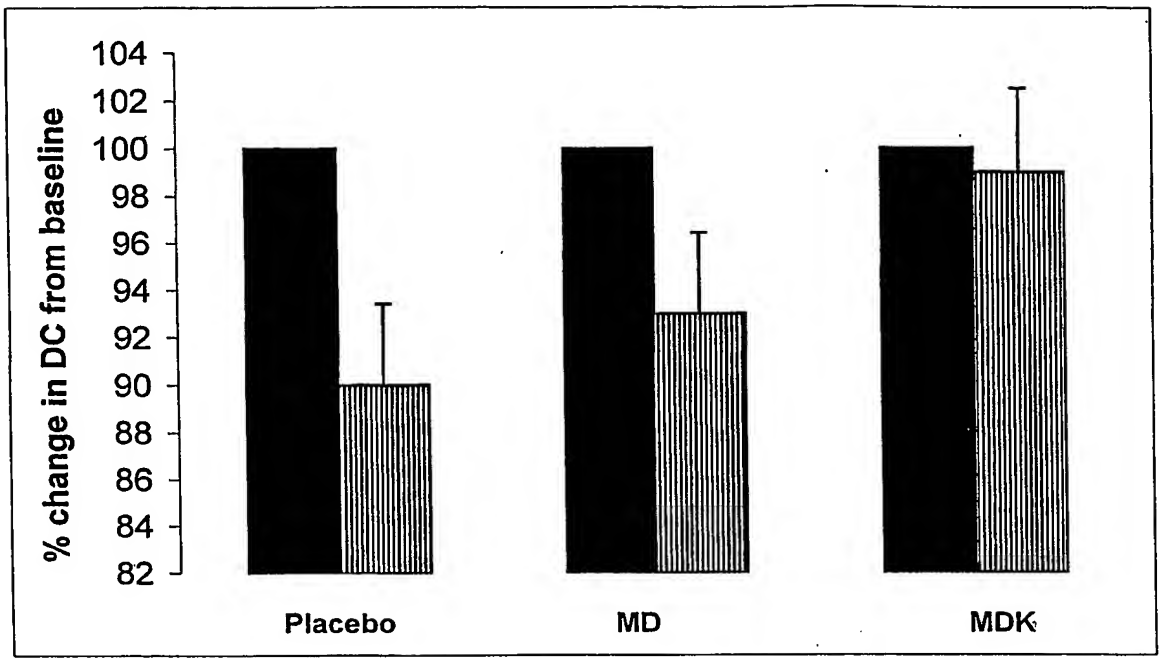
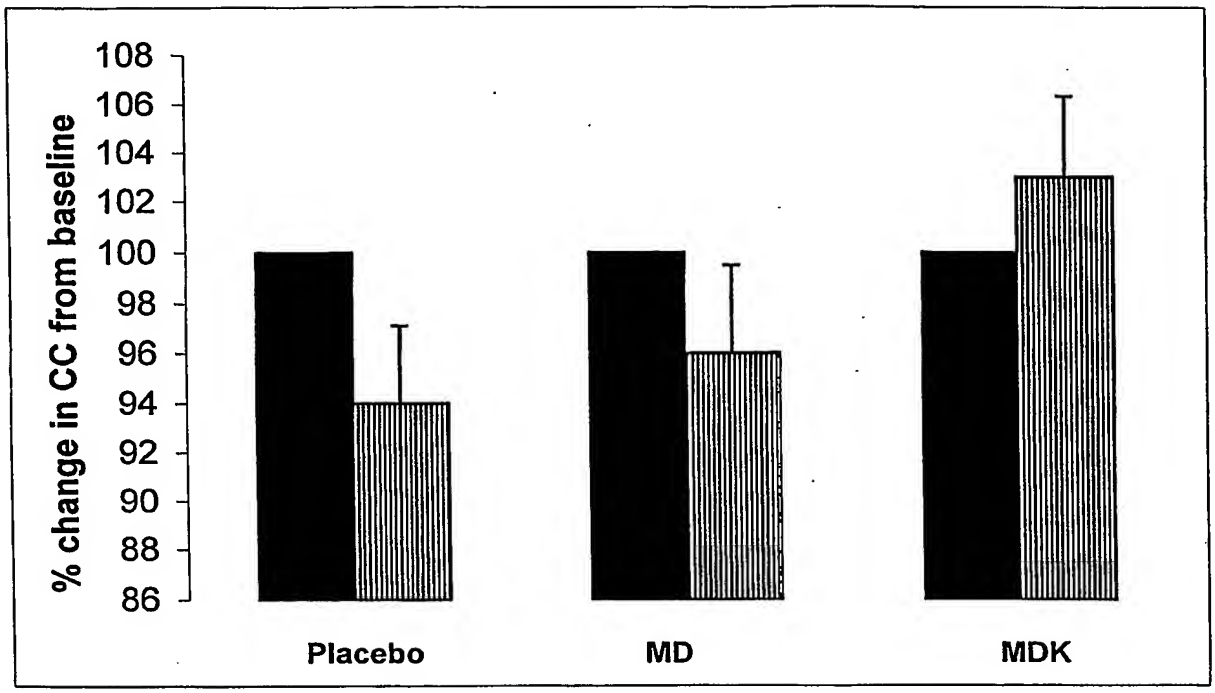

% change in DC from baseline
104
102
100
98
96
94
92
90
88
86
84
82
Placebo
MD
MDK

Fig. 2


% change in CC from baseline
108
106
104
102
100
98
96
94
92
90
88
86
Placebo
MD
MDK